

## Risk factors in the development of cervical intraepithelial neoplasia in women with vulval warts

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**SUMMARY** Of 59 women referred with vulval warts whose cervixes were assessed colposcopically for the presence of cervical intraepithelial neoplasia (CIN) before local treatment of the wart lesions, 17 had histologically proved CIN, 12 had histologically proved cervical wart virus infection, and 30 had abnormality on colposcopy or cytology. Seven of the 17 with CIN had no abnormality on cervical cytology.

No differences in sexual behaviour, smoking habit, or oral contraceptive use were seen between women with CIN and those with no cervical abnormality. Viral DNA typing of the vulval lesions was carried out, but there were no differences in the distribution of viral types between the three different histological groups.

Of the 30 women with no abnormality at the initial visit, 23 were followed up colposcopically and cytologically for one to two years. Three of them developed CIN after adequate treatment of the vulval lesions despite the absence of cervical abnormalities on colposcopy at the time of treatment.

Studying the known factors linked with CIN failed to show why some women with vulval warts develop CIN, even after treatment of the warts, and others do not. The large number of false negative results on cervical cytology in our patients suggests that women presenting with vulval warts should be screened colposcopically in the first instance. Close follow up of women whose warts are treated and who are thought to have no cervical abnormality at that assessment is essential.

Despite the circumstantial evidence implicating genital warts and infection with human papillomavirus (HPV) in the aetiology of cervical cancer,<sup>1,2</sup> not all women with genital warts develop cervical cancer. Prospective studies of women with cervical HPV infection have indicated that 12.1% to 13.4% of these lesions will progress to cervical intraepithelial neoplasia (CIN) over a variable time,<sup>3,4</sup> but there is little information on the risk of developing CIN in women presenting with overt vulval wart infection whose cervixes are not affected, and no data are available about the possible effects of treatment of the vulval lesions on the subsequent risk of CIN.

Walker *et al* showed that almost one third of women presenting with vulval warts would have histological evidence of CIN at initial assessment or within six

months of presentation.<sup>5</sup> Although follow up was short, three of the 22 women colposcopically healthy at their first visit developed colposcopic abnormalities within six months. Francheschi *et al* indicated that in women with genital warts who had normal cervical cytology and for whom further cervical smears were available, 10% developed an abnormal smear at a mean of three to four years.<sup>6</sup>

The progression of genital warts to cervical premalignant disease must therefore largely depend on other factors. Such external factors interact in other animal papillomaviruses, such as those of rabbits and cattle, to induce tumours.<sup>7,8</sup> Proposed cofactors in cervical cancer include cigarette smoking, prolonged oral contraceptive use, and type of HPV.<sup>9-13</sup>

In view of the large numbers of women with genital warts presenting to gynaecologists, dermatologists, genitourinary medicine specialists, and general practitioners, it would be useful to assess differences in the prevalence of these other risk factors in women with and without CIN at the time of initial presentation.

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This might permit the identification of high risk subgroups for whom intensive cytological or colposcopic surveillance would be worthwhile. Longer term follow up of women without cervical infection after being treated for vulval warts might give valuable information about the real day to day risks for women who develop CIN.

We therefore assessed, treated, and followed up for two years a group of women presenting with vulval warts.

## Patients and methods

### STUDY GROUP

The gynaecology, dermatology and genitourinary medicine clinics of Ninewells Hospital referred 59 women with vulval warts for colposcopic assessment. All the women gave full informed consent. For each woman we noted age, parity, marital status, number of cigarettes smoked and duration of smoking habit, contraceptive used and duration of use, age at first coitus, number of sexual partners, and history of genital warts in partners.

All women underwent colposcopic examination by one of us (JD). A cervical smear was taken using a modified Ayre's spatula. Directed biopsy specimens were taken of colposcopically abnormal epithelium, and a representative biopsy specimen of the cervical transformation zone was obtained from each woman without detectable abnormality. We used standard criteria for the histological diagnosis of CIN or HPV.<sup>14,15</sup>

Overt warts were sampled, and the tissue was snap frozen in liquid nitrogen. As the women had been referred initially for treatment of vulval warts, all were offered treatment with podophyllin, 5-fluoracil cream, or diathermy under general anaesthesia when appropriate. Women with histological evidence of

CIN were treated with the Semm cold coagulator.<sup>16</sup> All women with CIN were followed up at the cervix clinic at Ninewells Hospital. Women with benign histology results were reassured and asked to reattend one and two years after the initial examination when the colposcopic, cytological, and, when appropriate, histological assessments were repeated by the same assessor (JD).

### VIRAL DNA ANALYSIS

DNA was extracted in high volume 0.5% sodium dodecyl sulphate, 0.1 mol/l sodium chloride, and 0.05 trometamol (TRIS) hydrochloride (pH 8) containing 200 mg/l proteinase K (Boehringer); digested for 12–24 hours at 37°C; and then extracted using phenol and chloroform and stored in TE (TRIS 10 mmol/l and EDTA (ethylenediaminetetra-acetic acid) 1 mmol/l) buffer at –20°C.

Samples were digested with the bacterial restriction enzyme *Pst* I, the fragments were separated on agarose gels, and DNA was transferred to nitrocellulose filters as described previously.<sup>17</sup> The filters were hybridised at low stringency (melting temperature –40°C) with linear HPV DNA probes labelled with radiophosphorus, exposed to Fuji x ray film, and then washed at high stringency (melting temperature –12°C) before re-exposure. All samples were probed for the presence of HPV types 6, 11, and 16.

## Results

Of the 59 women referred, 17 had histological evidence of CIN (seven had CIN I, seven had CIN II, and three had CIN III), 12 had evidence of cervical HPV infection in the absence of CIN, and 30 had no cytological, colposcopic, or histological abnormality at the first visit.

Five of the 12 women with cervical HPV had normal

Table 1 Demographic data of 59 women with vulval warts

Patient characteristics	Cervical histology results showing:		
	CIN (n = 17)	HPV (n = 12)	No abnormality (n = 30)
Mean (SD) age (years)	22.7 (4.45)	22.4 (3.55)	25.7 (5.9)*
Mean (SD) parity	1.32 (2.18)	1.33 (1.82)	1.07 (1.33)
Mean (SD) age at first coitus (years)	17.1 (1.2)	16.7 (1.5)	18.0 (1.3)
Sexual partners: < 5	16	10	28
> 5	1	2	2
Mean (SD) duration oral contraceptive use (years)	1.56 (1.46)	4.4 (2.54)*	2.2 (2.01)
Smoking (total)	8	9	12
Smoking (pack years): < 2	1	1	2
3–4	4	5	2
> 4	3	3	8

CIN = cervical intraepithelial neoplasia.

HPV = human papillomavirus.

\*p < 0.05.

Pack years = Cigarettes/day × years ÷ 20.

Table 2 *Human papillomavirus (HPV) DNA types obtained from 56 women with vulval warts*

HPV DNA type	Cervical histology results showing:		
	CIN (n = 16)	HPV (n = 11)	No abnormality (n = 29)
No detectable DNA	8	6	16
6a	0	1	1
6b	1	0	0
6 (Dundee)	6	4	12
11	1	0	0
16	0	0	0

CIN = cervical intraepithelial neoplasia.

cervical cytology at the time of diagnosis. Seven of the 17 women with CIN, including two of the three with CIN III, also had normal cytology.

Table 1 shows the demographic data of the three groups. No differences in sexual behaviour were found between the groups. Women with vulval warts but normal cervixes were older than those in both groups with histological abnormalities ( $t = 1.93$ ,  $p < 0.1$  compared with women with CIN;  $t = 2.174$ ,  $p < 0.05$  compared with women with HPV). Most of the 59 women took oral contraceptive steroids. No differences in duration of their use were noted between women with CIN and those with normal cervixes, but those with cervical HPV only had used contraceptive steroids for significantly ( $p < 0.05$ ) longer than women in both the other groups. There were no differences in the proportion of smokers in the CIN and normal groups, although the women in the normal group tended to be heavier smokers. A higher proportion of women with cervical HPV smoked, but they were not heavier smokers than any women in the other two groups.

HPV DNA was extracted from vulval warts taken from 56 patients. Viral DNA was identified in 26, and table 2 shows the distribution of types. The virus named HPV (Dundee) has not yet been typed; the DNA hybridised to the HPV 6 probes under stringent conditions, but its restriction profile with the restriction enzyme *Pst* I differed from the known profiles of HPV types 6a, 6b, or 6c. It may represent a variant or a

possible DNA degradation problem in the extraction. HPV 16 was not identified in any wart tissue. HPV DNA was identified as often in the warts of women with healthy cervixes as in those with CIN or cervical HPV.

#### FOLLOW UP STUDIES

All women treated for CIN were followed up yearly at the cervix clinic at Ninewells Hospital. Treatment failed in one, who was treated again at one year.

Nine of the 12 women with cervical HPV attended for one year follow up. Cervical HPV persisted histologically in one, one had an atypical (class 2) smear with normal colposcopic findings, and the remaining seven had no colposcopic or cytological abnormality. Of the 30 women with normal findings at the initial visit, 18 attended for one year review; one had developed cervical HPV and one had developed CIN I, which was treated with the cold coagulator.

Five of the nine women with cervical HPV followed up at one year attended again for two year review. Two of them had developed CIN I, the HPV lesion persisting at year 1 had resolved, but the cytological abnormality in the other woman persisted, although her colposcopy result was again normal. Fourteen women initially normal were seen at two years (nine previously seen at year 1 plus five who did not attend at year 1). One had developed CIN I and one (normal at year 1) had developed CIN III. The cervical HPV lesion seen at year 1 had resolved, and one woman had an atypical (class 2) smear but normal colposcopy.

Table 3 shows details of the five women who developed CIN.

#### Discussion

Our finding of 17 women with established CIN at initial screening confirms the conclusions of Walker *et al* that women with genital warts are at high risk of having CIN.<sup>5</sup> These findings reinforce the view that women attending for the first time with genital warts required screening for cervical intraepithelial neoplasia. The type of screening is still controversial, but our rather alarming finding of many false negative

Table 3 *Characteristics of five women who developed cervical intraepithelial neoplasia (CIN) one or two years after being treated for vulval warts*

Age	Original histology	HPV type	Smoking (pack years)	Oral contraceptive use (years)
25	Normal	6a	0	5
21	Normal	Not tested	0	3
21	Normal	6 (Dundee)	0	4
21	HPV	Not tested	3-8	6
23	HPV	6 (Dundee)	6	8

HPV = human papillomavirus

Pack years = cigarettes/day  $\times$  years  $\div$  20.

results of cervical cytology (all in smears taken at the time of colposcopy) in a group of women with colposcopic and histological abnormality suggests that screening should be by colposcopy. The implications of such a policy would be profound, given the numbers of women to be screened. Screening of this nature would almost certainly need to take place in genitourinary medicine clinics and would require considerable financial and training resources.

We were unable to show any differences between women with CIN and those with normal cervixes at initial screening in respect of sexual behaviour, smoking habit, oral contraceptive use, or HPV type. This suggests that the factors that increase the risk of CIN in women with vulval warts have not yet been identified, although the small numbers in our study make any firm conclusions debatable. Walkinshaw *et al* who studied a larger group of women with CIN and cervical HPV, suggested that women with CIN who smoked were heavier smokers than those with HPV infection who smoked.<sup>18</sup> Our study did not confirm this finding. The larger study did not include data on duration of smoking. Other studies implicating oral contraceptives or smoking in cervical cancer used "normal" women as controls and did not give data on past or current genital warts.<sup>9-12</sup>

Lesions containing HPV 16 are more likely to progress to high grade CIN.<sup>13</sup> We were unable to identify this viral type in any specimen. Durst *et al* have shown that HPV 16 is rarely identified in vulval warts,<sup>19</sup> although Schneider *et al* identified HPV 16 more readily in condylomata and wart virus infected tissue in the absence of dysplasia.<sup>20</sup> HPV types 6 and 11 are commonly identified in vulval warts, and it is women with vulvar warts with HPV 6 and 11 who are said to be at risk of CIN. HPV of these types is found in CIN lesions, including those classified as CIN III.<sup>7,20,21</sup> No biopsy specimens from the cervix were HPV typed, so differences in cervical HPV isolation may have existed between our study groups as other studies have shown that HPV 16 is more common in CIN.<sup>22</sup> There is, however, a high correlation between the type of virus identified in lower genital warts and the viral type found in cervical lesions in the same woman.<sup>20</sup> Women in whom viral DNA was identified may therefore have had the same viral type in the cervical lesion. No differences in the incidence of the different viral types was seen between normal cervixes and those with histological abnormality.

Women with cervical HPV at initial screening were significantly younger than the normal group, had used oral contraceptive steroids for longer than both the normal and CIN groups, and were more likely to smoke than both the other groups. It is not clear why this group should have been different. One reason may be that prolonged exposure to oral contraceptives in

young women who smoke makes the cervix more susceptible to HPV infection. Why these differences should not be maintained in women with established CIN, however, is not clear.

The longer term follow up data gives cause for concern in women with genital warts. In all, 23 women initially normal colposcopically and cytologically were followed up at one or two years. All were treated for their vulval warts and were advised to encourage their sexual partners to seek examination and treatment. Three of them developed CIN, including one who developed CIN III. Even assuming that the women who did not attend for review remained free of CIN, this would represent a 10% progression rate to CIN in two years despite clinically adequate treatment of obvious lesions and no colposcopic evidence of cervical infection. The numbers studied were too small to show any differences in smoking, contraceptive use, or HPV type. The finding of two patients with cervical HPV infection developing CIN is consistent with previous studies.<sup>3,4</sup>

Women with overt genital warts represent a high risk group likely to have existing CIN when first presenting for treatment. CIN can develop despite adequate primary treatment of existing vulval lesions before any detectable cervical pathology. In this small study we have been unable to show any association between smoking, oral contraceptive use, or HPV type and the presence or development of CIN in women with vulval warts. We therefore suggest that women presenting for treatment of genital warts should be assessed colposcopically as well as cytologically. All histological abnormalities should be treated in view of the well established evidence on the progression of cervical HPV to CIN. In the absence of cytological and colposcopic abnormality at initial assessment, these women, even after treatment, should have yearly cervical cytology tests as a minimum follow up, and colposcopy at intervals may have to be considered. Large follow up studies of women treated for genital warts will be required to establish any possible links between progression and other potential aetiological factors.

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